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Recombination and Cellular Sensitivity to Radiation and
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13. ABSTRACT (Maximum 200 Words) The purpose of this research is to investigate the roles of RAD51-interacting breast cancer genes in homologous recombination and cellular resistance to anti-cancer drugs. We outlined 5 specific tasks in the original proposal. The first year of the project mainly involved tasks 1 and 5. Task 1 was designed to establish reliable over-expressing cell systems in mammalian cells. These cells will be used in tasks 2, 3, and 4 starting in the second year of the project. Without changing the objectives of the study, we have made improvement in the technical approaches toward the goal of this task, and significant progress was made in the first year of research. The purpose of task 5 was to identify novel BRCA interacting proteins. In the first year, we planned to complete the yeast two hybrid screen process, and we have accomplished it. Two new genes were identified by a yeast two-hybrid screening.				
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FOREWORD

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N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

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I. Introduction.

Studies have suggested that BRCA1 and BRCA2 may regulate homologous recombination through their association with RAD51 protein. We proposed to study the roles of BRCA genes in the regulation of DNA homologous recombination, and cellular sensitivity to some tumor therapy agents. To achieve these goals, we stated 5 specific tasks in the proposal. The first year of study mainly concentrated on the establishment of an over-expression system that can be used in mammalian cells. Without changing the tasks and objectives, we slightly modified the technical approaches to achieve higher scientific quality and be more cost-effective in task 1. The technical details for the rest of the tasks remain the same as proposed.

II. Body of Report.

1. Task 1(months 1-15).

We planed to use over-expressing approaches in a monkey cell line (FHS2) to investigate the roles of BRCA genes on spontaneous homologous recombination (task 2); in CHO cells (Strain Sce33) to study the effects of BRCA genes on double strand break (DSB) induced homologous recombination (task 3), and in human HT1080 cells to study the effect of BRCA genes on cellular sensitivity to drug (task 4). The majority of this task was proposed to be accomplished in the first year. Since the interaction between RAD51 and BRCA2 is better documented than BRCA1/RAD51 interaction, our work in the first year has been focused on the human BRCA2 gene, especially its RAD51-interacting domains.

In the original proposal, we planned to use CHO-Sce33 cells to study DSB-induced homologous recombination. Since the spontaneous recombination frequency in Sce33 cells is too low to detect, monkey FSH2 cells was chosen. Therefore, we had planned to establish expression systems for two different non-human cell lines. However, early in the first year of this project, we obtained a human cell line, HT1080-1853, from Dr. Mark Brennenman of Los Alamos National Laboratory. In this cell line, a recombination substrate using puromycin as the marker is integrated into a chromosome. It has a spontaneous recombination frequency of 10^{-05} , comparable to monkey FSH2 cells (Task 2). In addition, the structure and mechanism of DSB induction in HT1080-1853 is very similar to the ones in CHO-Sce33 cells. They have comparable DSB-induced homologous recombination frequency. Due to the advantage of human cells (HT1080) over monkey (FSH2) and hamster cells (CHO-Sce33) in studying the function of human genes, we decided to replace FSH2 (task 2) and CHO-Sce33 cells (task 3) with HT1080-1853 for our studies. Since the same cell line (HT1080-1853) can be used in tasks 2, 3, and 4, this replacement is more cost-effective without lowering the scientific objective. Our effort in the first year has been focusing on establishing an over-expressing system in HT1080-1853 cells.

We used the strategy outlined in our proposal to establish a stable cell line that can over-express BRCA2 fragments upon IPTG induction. Colonies isolated by this strategy has failed to produce IPTG-inducible BRCA2 fragments, presumably due to a growth inhibitory effect of the low level, background expression of BRCA2 fragments before the IPTG induction. This outcome was predicted by our original proposal (please see original proposal, Section E.4.1.d, Pitfall 2). Therefore, we decided to use the alternative approach proposed in our original proposal to circumvent this technical difficulty, by using a transient over-expression strategy. In such an

effort, we have created a vector named pEGES based on the pIRES vector (Clontech Laboratory).

pEGES contains an IRES element that enables two proteins be translated from a single mRNA molecule that was transcribed from the CMV promoter. Two multiple cloning sites (MCS) were available for subcloning. The first MCS site has a Nuclear Localization Signal tagged with HA-tag. Protein expressed from this site would be tagged with HA-NLS, and thus can be monitored with an anti-HA antibody, and be targeted into the nucleus. The second MCS site has a Flag epitope tag and a NLS signal sequence. Protein expressed from this site will also be targeted into the nucleus, and can be monitored by anti-Flag antibody. Under the control of another promoter (pSV40), the EGFP gene was cloned in. EGFP expression is used to monitor the transfection efficiency of the transient expression.

Three RAD51-interacting domains of BRCA2 were individually cloned into the first MCS site, including the BRC repeats 7 and 8, and a C-terminus domain. The I-SceI gene was cloned into the second expressing site. Therefore, transient expression of a RAD51-interacting domain and the I-SceI enzyme can be accomplished in the same cell simultaneously. This approach reduces the risk of potential growth arrest in HT1080 cells as opposed to stable transfection approaches. Currently, using GenePorter (GeneTherapy System) or Lipofectamin (Gibco) transfection techniques, we routinely obtain 40-60% expression efficiency from the transfected plasmids as measured by EGFP signal. We will use flowcytometry technology to sort EGFP-positive cells for the recombination assay, which will further improve the assay.

With such improvements, we will be able to study the effects of transient over-expression of RAD51-interacting domains of BRCA2 in spontaneous recombination (task-2), site-specific double strand break induced homologous recombination (Task-3), and cellular sensitivity to tumor therapy agents. We anticipate it will produce desired results by months 15.

By summary, although we had to use the alternative approach outlined in the original proposal to achieve the goal of over-expression of BRCA2 gene fragments, we have achieved the scientific goals proposed for the first year in Task 1. The adjustments and improvement of the system should improve the scientific quality of our works in tasks 2, 3, and 4 that would be started in the second year.

2.Task 2. Spontaneous recombination (Months 13-30).

Task 2 will be started in the second year as proposed. As discussed above, we will use the transient over-expression system in HT1080-1853.

3. Task 3(months 12-30):

We have started this task by introducing the transient expression vectors into the HT1080-1853 to study the effect of RAD51-interacting domains of BRCA2 in the regulation of DSB-induced homologous recombination.

4. Task 4(months 12-30).

We have started this task by introducing the transient expression vectors into the HT1080-1853 cells. We will test 1-2 tumor therapy drugs at first.

5. Task 5.

We proposed to identify novel BRCA interacting proteins. In the first 12 months of the project, we have accomplished the work for task 5 (items 1 and 2), and successfully isolated two

independent cDNAs that code for proteins interacting with BRCA2 in yeast two-hybrid system. We expect to characterize the cDNA clones as planned in the Statement of Work in the second year of the research.

III. Key Research Accomplishments in the first year.

1. Accomplished the work outlined in task 1, with improvement in the expression cell system that would improve the scientific quality for task 2, 3, and 4. This includes the creation of pEGES vector, and establishment of BRCA2 over-expression system in HT1080 cells.
2. Identified 2 novel cDNAs coding for proteins that interact with the C-terminus region of BRCA2 proteins.

IV. Reportable Outcomes.

1. **Manuscripts, abstracts, presentation:**
None.
2. **Patents and Licences applied for and/or issued**
None.
3. **Degrees obtained**
Not applicable.
4. **Development of cell lines, tissue or serum repositories:**
None.
5. **Informatics such as databases and animal models.**
Not applicable.
6. **Funding applied for based on work supported by this award.**
None.
7. **Employment or research opportunities.**
Not applicable.

V. Conclusion.

- A transient over-expression system that enables co-expression of BRCA2 fragments and the DSB-inducing I-SceI enzyme is being established in human cell. This system will significantly facilitate our work towards task 2, 3, and 4.
- Human HT1080 cells will be used as the primary system to achieve the objective outlined in the proposal. Using human cells, instead of monkey and CHO cells will provide more reliable information about human BRCA genes and their role in the regulation of DNA recombination and response to tumor therapy agents.
- The RAD51-interacting domains of BRCA2 have been chosen as our priority in the next year's study.
- Significant progress was made toward the objective of task 5. We have isolated two novel genes whose protein products interact with BRCA2 in a yeast two hybrid system. We will concentrate on the characterization of these interactions in the coming years.

VI. Reference.

None.

VII. Appendices.

None.